05-24-02

1634

#10/a

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	MANSOUR SAMADPOUR
SERIAL NUMBER/FILING DATE:	09/380,422
TITLE OF INVENTION:	METHOD FOR THE IDENTIFICATION OF GENETIC SUBTYPES
EXAMINER/ART UNIT:	Frank W Lu/ Unit 1655
ATTORNEY DOCKET NUMBER:	SAM23Pat

Commissioner of Patents and Trademarks
Box 5
Washington D.C. 20231

Sir:

The following amendments, new claims and remarks are in response to Office actions, mailed: June 6,2001 and April 23,2002. In a telephone conference with the examiner, it was learned that the figures 1 -16 have been located and are now with the application.

Please amend the above application as follows.:

in the abstract:

1. Please add the following abstract (clean version) to the application as required by the examiner. A clean version on a separate page as required is submitted following this response.

"Abstract

The invention provides a method for the identification of genetic subtypes using restriction endonuclease analysis of genomic DNA followed by resolving the DNA fragments by electrophoresis. The method employs frequent cutting restriction enzymes that produce large number of fragments between 0.1 to 18 kb. The resulting restriction fragment polymorphism patterns are highly differentiative and allow for grouping the microbial strains into groups of clonal origin."

in the specification

2. Please add to page 1, after the section entitled: FIELD OF INVENTION and before BACKGROUND,the following section:

"PRIOR PROVISIONAL PATENTS"

"The inventor claims benefit of the provisional co-pending patent application entitled **MICRO-RESTRICTION RESTRICTION FRAGMENT LENGTH POLYMORPHISM** filed by Mansour Samadpour on March 10,1997 with the U. S. Patent Office, number 60/~~167734~~ 039,056."

In the Claims:

3. Please amend claim 16 as follows (clean version, marked up version follows remarks)

claim 16. (amended) A method for the interstrain differentiation of bacterial isolates comprising the steps of:

i. isolating genomic DNA from
   a) a panel of epidemiologically linked bacterial isolates and
   b) a panel of epidemiologically un-linked bacterial isolates wherein said linked and un-linked isolates are members of the same genus;

ii. restricting genomic DNA from the linked and un-linked panel of isolates with a panel of restriction enzymes selected from the group consisting of AccI, AIuI, AvaI, AvaII, BanII, Bfal, CfoI, DdeI, DpnI, HaeII, HaeIII, HhaI, HincII, HinfI, HpaII, MboI, MnII, MseI, NciI, NlaIV, RsaI, Sau3AI, ScrfI, TaqI, and ThaI;

iii. resolving the restricted DNA of step (ii) by gel electrophoresis to generate a banding pattern for each enzyme;

iv. selecting at least one enzyme from the panel of enzymes of step (ii) that
   a) restricts all the genomic DNA to fragments of less than 18 kb,
   b) generates a lowest index of diversity from the linked panel of the isolates selected and
   c) generates a highest index of diversity from the unlinked panel of the isolates selected;

v. restricting the isolated DNA from the linked and un-linked panels of bacterial isolates of step (i) with the at least one

enzyme of step (iv);

vi. resolving said restricted DNA of step (v) by gel electrophoretic means capable of separation fragments in the 0.1 to 18 kb range to generate a distinct genetic fingerprint for said linked and unlinked isolates; and

vii. comparing said distinct genetic fingerprints of step (vi) wherein differences in said genetic fingerprints afford interstrain differentiation of said bacterial isolates.

Q3

REMARKS

1. The specification has been amended to comply with the examiner's requirement that the benefit of priority to the provisional application as described by the applicant in his declaration and power of attorney filed with the application, be incorporated as part of the specification. No new matter is added by this amendment. A marked up version of this amendment is submitted following the remarks portion of this paper.

2. Claim 16 has been amended to define the invention more particularly and distinctly as a result of the examiner's comments regarding indefinite terms of "highest index" and "lowest index." The amendment clarifies that these terms are defined "within all enzymes selected."

3. Section 112 rejection of claims 7,8,10 and 17

The examiner rejected claims 7,8,10 and 17 for failure to enable a person skilled in the art to show the separation of digested small DNA fragments such as 0.1 - 0.2 kb using pulsed field electrophoresis and the separation of digested large DNA fragments such as 10 -18 kb using capillary gel electrophoresis. The examiner notes that he could not find a prior art that describes the separation of digested DNA fragments from .1 to 1 kb using pulsed field electrophoresis or the separation of digested DNA fragments from 10 to 18 kb using capillary gel electrophoresis concluding that undue experimentation is required for the invention.

Reconsideration of the examiner's action is respectfully requested. Claim 7 adds the limitations of a gel electrophoresis process to the limitations of claim 6. Claim 6 adds limitations of the chromatographic means of step iii of independent claim 1. Claim 8 limits the gel limitations of claim 7 to an agarose gel having a specific concentration of about 0.6% to about 2% agarose. Claim 10 limits the type of staining of claim 7 to a specific choice of dyes. Claim 17 limits the methods of gel electrophoresis to a choice described therein as further limitations to independent claim 16.

It is well known that the choice of electrophoresis method employed in resolving DNA fragments depends on the size range for the restricted fragments. It is equally well known that Although the method of pulsed field gel electrophorsis ("PFGE") is used for resolving very large size DNA fragments (10kb to >1000 kb), it can also be used for resolving smaller fragment ranges. The reason that it is not used often for resolving small size ranges is the expense and time associated with the use of PFGE does not justify its use, where the same can be accomplished by conventional agarose gel electrophoresis.

Furthermore, to a person learned in the art it is obvious that capillary gel electrophoresis is used for resolving small DNA fragment size ranges. However, while capillary gel electrophoresis is currently in use for resolving small fragments (up to 2-4 kb), there upper end of the size range is constantly expanded, but even now some of the DNA ranges (.1-4 kb) generated by MRF can be resolved by capillary gel electrophoresis.

Claims 7, 8, and 10 refer to the use of electrophoresis methods for resolving the MRF generated fragments. As can be appreciated, the methods of Micro Restriction Fingerprinting (MRF) is used to cut the genomic DNA into fragments ranging from 0.1 to 18 KB. The exact size range depends on the specific genomic DNA and the restriction enzyme. While the lower size range is approximately 0.1 kb range the upper size could vary from a few kilobases to 18 kb. In any MRF subtyping all or a portion of the resulting

fragments can be analyzed to identify genetic subtypes. Claims 7, 8, and 10 refer to the use of electrophoresis methods for enhancing the resolution of MRF generated fragments. The various gel electrophoresis methods are well known to persons skilled in this art. Examples of these methods include but are not limited to pulsed gel electrophoresis ("PFGE"), horizontal gel electrophoresis, vertical gel electrophoresis, field inversion gel electrophoresis, and capillary gel electrophoresis. The choice of the method depends on the fragment size range that are to be resolved. For example, horizontal gel electrophoresis, vertical gel electrophoresis, PFGE, and field inversion can be used to resolve the entire size range, while capillary gel electrophoresis can be used to resolve more limited, smaller size range. The most commonly used PFGE equipment sold by Bio Rad, Cheff Mapper, is used to resolve fragments of any size range. PFGE reliably resolves large fragment size ranges to small fragment size ranges. Likewise, capillary electrophoresis is used to reliable resolve fragments for RFLP patterns in the lower size ranges.

Choosing a method to resolve various size fragments is well known to persons skilled in this art and is part of the standard practice. It is well known that capillary gel electrophoresis is used for resolving smaller size fragments while PFGE and field inversion is used for larger size ranges while one dimensional gel electrophoresis is used for small to medium size range fragments. A person skilled in this art is familiar with choosing the reliable technique to be used according to the size fragments.

The limitations of Claim 10 is directed to the use of various dyes for staining the DNA fragments to enhance visibility of the resulting bands. A person skilled in the art is familiar with the use of DNA staining protocols for enhancing visibility of DNA fragments.

It is argued that it is well known for a person skilled in this art that the choice of the alternative method limitations of these claims depends upon the size of object fragments. Each method is well known as it relates to resolution according to fragment

size. Accordingly, the limitations of choices described in these claims are enabling and truly define a patentable invention.

4. § 112 rejection of claims 16 - 27.

The examiner rejects claims 16 -27 as being indefinite, further noting that the phrases "lowest index of diversity" and "the highest index of diversity" are unclear, not understood and are relative terms. Furthermore, the examiner did not understand the exact meaning of "index of diversity" albeit index of diversity is defined in the specification.

Reconsideration of the examiner's action is respectfully requested. The terms "lowest index of diversity" and "highest index of diversity" in the context of this art are well known to a person of ordinary skill. These terms refer to the step of comparing a set of information to another set of information and extracting information having a quantitative maximum and minimum. Both sets of information well defined. The comparison is well defined and unambiguous. The terms "highest" and "lowest" are well known terms of this art that require the user to find an isolate that provides for the highest differentiative power for the epidemiologically unrelated collection of isolates compared with the epidemiologically related isolates.

Independent claim 16 provides steps for the method for interstrain diffentiation of bacterial isolates. Claim 17-27 provide additional limitations to these steps to further define the invention. Dependent claim 17 requires that method of gel electrophoresis be selected from a group of known fragment separator techniques to resolve RFLP patterns. Dependent claims 18 and 19 further limits claim 16 as to the type of gel used in the gel electrophoresis step 16(iii). Dependent claim 20 provides a dye means to resolve the restricted DNA of claim 16. Dependent claims 21 and 22 further provide limits to selection of enzymes referred to in step ii of claim 16.

Claim 23 provides limitations to the genomic DNA. Claims 24 and 25 provides limitations to step iii of claim 16. Claim 26 limits the bacterial isolate in the preamble of claim 16 to a

member of the group defined by this claim and Claim 27 further limits the preamble of claim 16 to the bacterial isolate to E. coli 0157 and Salmonella.

An average molecular epidemiologist skilled in this art understands the terms "highest" and "lowest" index of diversity in the context of these claims. The terms "lowest index of diversity" and "the highest index of diversity" are used in reference to the comparison of two sets of results to describe how the restriction patterns or banding patterns are analyzed to identify the enzymes that give the lowest, or least index of diversity from the linked panel of organisms, and the highest or most index of diversity from the unlinked panel. (page 12, lines 10- 14).

The specification further describes that the index of diversity is determined by dividing the number of banding patterns over the number of organisms tested, explaining that a pattern is considered significant if all the bands generated are less than 18 kb and the discrete bands are identified. (Page 12, lines 14 - 15).

To determine the most suitable restriction enzyme for an unknown organism to be subtyped by MRF, the invention assembles two collections of isolates from the species in question. By way of a typical example, assume the assembly of 20 isolates of an unknown species is taken from a cluster of cases in a waterborne outbreak. This assembly is the collection of epidemiologically linked isolates. The method requires that an assembly of a collection of isolates with no apparent epidemiological linkage to the outbreak be compared to this assembly. That is, an assembly taken at different times, from different sources and at different places. This is the unlinked collection of isolates. The method describes that both collections are to be subtyped with a battery of 20 restriction enzymes. The method requires a search for the enzyme(s) that provide the greatest or highest index of diversity for the collection of unrelated isolates, as well as the least or lowest index of diversity for the collection of linked isolates. That would be an enzyme that would divide the 20 unlinked isolates into 20 groups, giving the index of diversity of 1, that is the number

of unlinked isolates 20 divided by the number of groups 20 or 1. For linked isolates from the same outbreak, the RFKP pattern will show identical RFLP pattern for the 20 isolates since they are from the same outbreak. The ratio of index of diversity would show only one RFLP pattern, thus it would have an index of diversity of 1, that is the number of distinct isolate patterns divided by the 20 groups, or 0.05. Obviously, the range for the possible index of diversity may vary only from 0 to 1.0.

Accordingly, the limitations "lowest index" and "highest index" are not unbounded, vague, indefinite nor ambiguous. For an ordinary molecular microbiologist skilled in this art the terms "index of diversity", "lowest index of diversity" and "highest index of diversity" are terms well defined and used to comparing isolates in two different groups.

In the alternative and in the event that the above argument is not persuasive, step iv of claim 16 has been amended to define the invention more particularly and distinctly as a result of the examiner's observation that the terms "lowest index of diversity" and "highest index of diversity" are indefinite as originally drafted. The relevant portions of amended claims add the words " <u>of the isolates</u>" to follow the objected phrases so those portions of independent claim 16 reads as follows:

iv.....

b) generates a lowest index of diversity from the linked panel of <u>the isolates</u> selected and

c) generates a highest index of diversity from the unlinked panel of <u>the isolates</u> selected;

The amendment is directed towards providing additional structural limitations for the terms lowest and highest to clearly define that these terms are limited to only the comparisons to be made within the set of enzymes selected in (ii) for this step. Claims 17 - 27 are dependent upon claim 16(as amended) and are now clearly defined.

5. § 102 rejection of claims 1-4,6,11,12,16,19,21,25 and 26.

The examiner rejects claims 1-4,6,11,12,16,19,21,25 and 26 as

anticipated by Preston, et al (J. Clin. Microbiology, 32, 1427-1430, 1994)

Reconsideration of the examiner's action is respectfully requested.

Preston et al. (1994) ("Preston") does not anticipate the instant invention. Preston uses a *Hae* III to generate silver stained Restrictive Fragment Length Polymorphism ("RFLP") patterns for *Shigella sonnei*. The size range of the patterns are from .2-4.3 kb. Preston does not resolve the fragments using agarose gel electrophoresis, nor PFGE. This reference does not stain the fragments using ethidium bromide, propidium iodide, and fluorescent dyes.

Preston visualizes banding pattern based only on silver staining which is a complicated staining process, that takes 4-6 hours using a variety of toxic chemicals (see for example Page 1428, first column of Preston). The instant invention uses a dye enhancing means that takes only from 10-20 minutes to visualize. Furthermore, the quality of the banding pattern generated by the Preston reference is so poor that this method is not widely adopted in the microbiology community due to difficulties in analysis based on the poor quality of the resulting RFLP patterns. With the MRF criteria stated in the instant invention neither *Hae*III nor *Rsa*I qualify as the a selected enzyme for *Shigella sonnei*. The instant invention identifies *Sau*3A as the enzyme of choice for *S. sonnei* (see page 19 of the application #10, and figure 3B.)

The advance by the instant invention over the cited reference can be appreciated by a comparison of the methodologies and the improvements made: If a collection of S. sonnei isolates were subtyped, the enzyme reaction would have been set using 10 times more DNA than used by the Preston method. The enzyme used would have been Sau 3A instead of Hae III. The restricted DNA would have been resolved by agarose gel electrophoresis instead of polyacrylamide, and the gel would have been stained using a fluorescent dye instead of the silver stain. The size range would have been .1-13 kb instead of .2-4.3. The result of the instant

invention is much easier to analyze because of the quality of the banding pattern are bright well separated bands as compared to the results using the Preston method. Preston did not anticipate the methods described by the instant invention.

As an aside, a comparison of the poor quality of the RFLP patterns generated by the Preston method with the highly defined patterns generated by the instant invention underscores the advances made by the instant invention as well as explains why the Preston method thou cited by the examiner, has not been adapted for use in the field. The improved MRF method described in the instant invention that generates patterns having high differential power is an unanticipated advance over the Preston patterns. The improved MRF is an improvement over the Preston method as a universal typing method for a large number of gram positive and gram negative bacteria. The instant invention is not anticipated by the RFLP patterns described in the Preston reference; there is nothing in common between these methods. Lastly, in spite of the availability of the Preston reference, until MRF, there existed a continuing need for a simple and straight-forward chromosomal based subtyping method adaptable by persons skilled in this art. The Preston reference does not supply this need.

6. § 103 rejection of claims 5, 14, 15, 18, 24, and 27.

The examiner rejects claims 5, 14, 15, 18, 24, and 27 as being obvious over Preston et al.(1994) ("Preston") as applied to claims 1-4,6,11,12,16,19,21,25 and 26 above and further in view of Samadpour et al (j. Clin. Microbiol 31.3179-3183, Dec, 1993)("Samadpour").[1]

Reconsideration of the examiner's action is respectfully requested.

---

[1] The last two lines of Page 7 of the office action appears to be out of context with the text ending two lines separated by two empty lines above. It appears to the undersigned that the text continues from the words ", which encompasses all: with the text of page 8; the last two lines of page 7 are assumed to not be part of the office action.

As explained in In re Reinhart, 531 F.2d 1048, 1890 USPQ 143, 147 (CCPA,1976) in order to make a prima facie case of obviousness, the examiner must explain why the prior art would appear to show the claimed subject matter and not simply suggest that the general aspects of the invention are obvious in view of the art cited by the examiner. When more than one reference or source of prior are is required in establishing obviousness as a basis for rejection, it is necessary for the examiner to ascertain whether the prior art teaching would appear to be sufficient for one of ordinary skill in the art, to suggest making the claimed substitution or other modification. In re Lalu, 747 F. 2d. 703, 223 USPQ 1257, 1258 (Fed Cir. 1984); In re Fine, 837 F.2d 1071, 5 USPQ 2d 1596, 1598 (Fed Cir. 1988).

The arguments regarding the difference between the instant invention and the Preston reference of the previous request for reconsideration are incorporated herein by reference. The Preston reference describes using "Conventional typing" by using the method described by Szturn-Rubinsten in a 1968 publication to determine Genetic Variability and Molecular Typing of Shigella sonnei Strains Isolated in Canada. There is nothing in this reference that anticipates or makes obvious any of the teachings or claims of the instant invention. While Preston discusses subtyping of Shigella, one of the organisms listed in claims 4 and 26, Preston does not discuss any of the unique aspects of the method for differentiation of the genetic sub-types within wide variety of distinct organisms taught by the instant invention. As discussed before, Preston's method for subtyping Shigella sonni, is based on the use of a wrong enzyme, different size range, only one species of bacteria, different concentration of DNA, and a cumbersome DNA staining method, which in itself makes the method hard to perform and obsolete. None of the elements of the instant invention could have been predicted by the Preston's teachings.

The reference to Nucleic acid hybridization: A practical approach, Hames and Higgins, 1985, pages 1456 and 146) ("Hames") is inappropriate. The reference protocol is for isolation and

purification of DNA fragments (for making DNA probes or for cloning) using a preparative agarose or polyacrylamide gels. The width of the wells in preparative gels are 10-20 times that of analytical gels, that is how 10-50 micrograms of restricted DNA can be loaded on such gels without overloading the gels. This has nothing to do with the use of the 5-30 micrograms of DNA in MRF in wells which are 10-20 times narrower than the preparative gels. Because of the significance in the difference in the loading of the gels, there is no basis to find that it is obvious to an ordinary person skilled in the art to read Hames and find the instant invention equivalent or structurally similar.

The Samadpour reference does not anticipate claims 13 and 14. Samadpour uses one microgram of DNA per reaction, as opposed to 5-30 micrograms in the instant invention. Using one microgram for MRF will result in a RFLP pattern that would be not be visualized. Since the method used in the Samadpour reference generates a large number of small fragments, there are simply not enough of each of the fragments to allow for visualization using any of the staining protocols for agarose gels. The enzymes used by Samadpour are six base cutters in individual reactions. These enzymes can not be used in MRF since they generated fragments in 1-50 kb size range, which makes it impossible to analyze the resulting RFLP patterns (A very compact cluster of bands that often shows as a smear). That is why the method in Samadpour resorts to the use of southern blotting with subsequent probing using radioactive labeled lambda DNA probe to obtain a useful RFLP pattern. This is counter intuitive and not within the teachings of the instant invention.

The instant invention avoids the need for southern blotting and probing by generating a differentiative RFLP pattern by use of simple restriction enzyme reactions followed by simple agarose gel electrophoresis. There is nothing in the Preston reference, alone or in view of Samadpour in combination that anticipates or makes obvious the methods described in the instant invention.

It is respectfully submitted that the examiner lacks a basis to argue that the MRF method assumes a simple substitution of

organism (E. coli instead of Shigella) and substitution of polyacrylamide with agarose. There is nothing in the references cited by the examiner to support this assertion or suggests this substitution. It is improper to apply the "obvious to try" rationale to support an obviousness rejection. In In re O'Farrell, 853 F.2d 894, 903, 7 USPQ2d, 1673, 1681 (Fed Cir 1988), the court held that a claimed method may be obvious over the prior art relied upon when one reference contains a detailed enabling methodology, a suggestion to modify the prior art to produce the claimed invention, and evidence exists suggesting the modification would be successful. It is argued that in the present circumstances, the examiner lacks support to assert that the substitutions suggested by the examiner are supported by the references cited.

It is noted that the MRF method of this invention appears universal to bacterial species, the method is based on the use of class of enzymes or combination of enzymes that can generated fragments below 18 kb to allow for effective separation of the fragments using agarose gel electrophoresis or other forms of electrophoresis. The instant invention employs rapid and simple staining protocols an advance above the methods described in Preston. It also improves on the Samadpour method by eliminating the need for southern blotting and hybridization with DNA probes. As described in the specification, the invention describes a universal subtyping methodology as an alternative to PFGE subtyping methods described in the references cited. Unlike the PFGE method the instant invention does not require the use of expensive specialized electrophoresis equipment that cost from $10,000-$20,000.

7. § 103(a) rejection of claims 7,9,10,17 and 20.

The examiner rejects claims 7,9,10,17 and 20 as anticipated by Preston, et al (J. Clin. Microbiology, 32, 1427-1430, 1994) in view of Arakawa et al (J. Chromatography A. 664, 89 - 98, 1994)("Arakawa"). Reconsideration of this rejection is respectfully requested because it is submitted that there is no reason suggested in the references themselves to make the suggested

combination resulting in the instant invention.

Arakawa's reference is inapplicable. Arakawa's reference discusses Capillary Gel Electrophoresis ("CGE") for the analysis of Polymerase Chain Reaction ("PCR") There is no relationship between the ability to separate and detect PCR amplicons and that of genomic DNA based RFLP banding pattern as suggested by the examiner. PCR methods deal with DNA fragments that are often in .1-1 kb range, vs. genomic DNA fingerprints in which a chromosome of 5,000,000 kb is restricted into thousands of fragments and the fragments are resolved and analyzed. Arakawa's work is completely distinct. The instant invention does not claim nor describe the use of ethidium bromide and the UV light for detecting DNA fragments, the invention describes is for their use (and use of similar dyes) in visualizing the MRF-RFLP pattern. Previous to the instant invention, it was impossible to generate and visualize chromosomal RFLP patterns in <18kb size range using these stains. There is no suggestion by Arakawa, directly or indirectly of the suitability of substituting polyacrylamide gel with capillary gel. Furthermore, no claim by the inventor is made to this process; the claim is to a step in the method of identification of genetic subtypes.

As discussed in the specification:

> "...Polyacrylamie gel separation is necessary to achieve effective separation in the lower size range which has very limited use for interstrain differentiation." (page 2, lines 29- 30)

This is contrary to the suggestion by the examiner. The applicant finds that polyacrylamie gel separation <u>is necessary</u> and not the same as capillary gel separation method. The examiner lacks foundation to assume that the capillary gel separation and polyacrylamie gel separation methods are the same or behave identically because they are both well known. The examiner requires additional support to demonstrate a result contrary to the teachings of the instant invention which makes polyacrylame gel separation necessary.

7. § 103(a) rejection of claims 7,9,10,17 and 20.

The examiner rejects claims 7,9,10,17 and 20 as anticipated by Preston, et al (J. Clin. Microbiology, 32, 1427-1430, 1994) in view of Arakawa et al (J. Chromatography A. 664, 89 - 98, 1994)("Arakawa"). Reconsideration of this rejection is respectfully requested because it is submitted that there is no reason suggested in the references themselves to make the suggested combination resulting in the instant invention.

Arakawa's reference is inapplicable. Arakawa's reference discusses Capillary Gel Electrophoresis ("CGE") for the analysis of Polymerase Chain Reaction ("PCR") amplicons. There is no relationship between the ability to separate and detect PCR amplicons and that of genomic DNA based RFLP banding pattern generated by the instant invention, as suggested by the examiner. PCR methods deal with DNA fragments that are often in .1-1 kb range, vs. genomic DNA fingerprints in which a chromosome of 5,000,000 kb is restricted into thousands of fragments and the fragments are resolved and analyzed. Arakawa's work is completely distinct. The instant invention does not claim nor describe the use of ethidium bromide and the UV light for detecting DNA fragments, the invention describes is for their use (and use of similar dyes) in visualizing the MRF-RFLP pattern. Previous to the instant invention, it was impossible to generate and visualize chromosomal RFLP patterns in .1 - 18kb size range using these stains, due to the insufficient quantities of DNA which was used in the restriction digestion reactions. There is no suggestion by Arakawa, directly or indirectly of the suitability of substituting polyacrylamide gel with capillary gel. Furthermore, no claim by the inventor is made to this process; the claim is to a step in the method of identification of genetic subtypes.

As discussed in the specification:

> "...Polyacrylamie gel separation is necessary to achieve effective separation in the lower size range which has very limited use for interstrain differentiation." (page 2, lines 29- 30)

This is contrary to the suggestion by the examiner. The applicant finds that polyacrylamie gel separation is necessary and not the same as capillary gel separation method. The examiner lacks foundation to assume that the capillary gel separation and polyacrylamie gel separation methods are the same or behave identically because they are both well known. The examiner requires additional support to demonstrate a result contrary to the teachings of the instant invention which makes polyacrylame gel separation necessary.

8. § 103(a) rejection of claims 13 and 22

The examiner rejects claims 13 and 22 as applied to claims 1-4, 6,11,12,16, 19, 21,15 and 26 and further in view of Clayton et al (J. Clin Microbiology, 31, 1420 - 1425, June 1993) ("Clayton") and Stratagene Catalogue (1994, page 211), ("Stratagene") . Reconsideration of this rejection is respectfully requested because it is submitted that there is no reason suggested in the references themselves to make the suggested combination resulting in the instant invention.

Stratagene reference lists restriction enzymes available for purchase in terms of name, size, catalog and price. (Quick list)

Distinction should be made between the methods used for separating PCR amplicons and RFLP analysis of genomic DNA. Clayton teaches the use of a single or double digestion for cutting small PCR amplicons into a few fragments to allow for identifying any differences in these short pieces of DNA. Claims 13 and 22 refer to the use of at least two 6 base cutters as a restriction enzyme as described in claim 1(ii). Restriction enzyme digestion of an entire chromosome is at least a 1000 times larger than the PCR amplicons that were analyzed by Clayton. There is no basis, as suggested by the examiner to expect that the use of restriction enzyme digestion to separate small PCR amplicons into fragments would have the same expectation for use in an entire chromosome having a length three orders of magnitude larger than the PCR amplicons. This expectation is not revealed in Clayton, Preston or in the Stratagene references.

The invention claims a means for visualizing very large and complex RFLP patterns in a fast, reliable manner. The use of at least two 6 base cutters for genomic DNA as a step in differentiating genetic sub-types within a population of genetically related organisms is not suggested, discussed nor anticipated by Clayton or Preston, alone or in combination. While Stratagene provides a catalog for restriction enzymes, there is no suggestion for their uses. Lastly, no claim is made for the multiple enzyme digestion method by the applicant. Rather, the inventor finds a new use for multiple enzyme digestion that produces an unexpected result when applied to chromosomal DNA, by producing an RFLP pattern that divides the isolates into groups of clonal origin. There is no suggestion made by Preston, Clayton, or the Stratagene catalog of this finding.

As was stated in In re Sernaker, 217 U.S.P.Q. 1, 6 (CAFC 1983),

> "(P)rior art references in combination do not make an invention obvious unless something in the prior art references would suggest that advantages to be derived from combining their teaching."

As was further stated in Orthopedic Equipment co. Inc. v. United States, 217 U.S.P.Q. 193, 199 (CAFC 1983),

> "It is wrong to use the patent in suit (here the patent application) as a guide through the maze of prior art references, combining the right references in the right way to achieve the results of claims in suit (here the claims in issue). Monday morning quarterbacking is quite improper when resolving the question of nonobviousness in a court of law (here the PTO)."

Conclusion

For all the reasons given above, this application is now submitted to contain claims which define a novel, patentable, and truly valuable invention. Hence allowance of this application, as amended is respectfully submitted to be proper and is respectfully solicited. In view of the above remarks, reconsideration and allowance of the claims are respectfully requested.

Should there be any remaining issues or topics which should be addressed, the applicant respectfully requests that the examiner contact the undersigned, by telephone: (206) 361-4840.

May 23, 2002

Joseph Chalverus, 30144
Attorney for Applicant

EXPRESS MAIL CERTIFICATE

EXPRESS MAIL CERTIFICATE NUMBER: ET 437864836 US

I certify that this present document is being deposited with the United States Postal Service "Express Mail Post Office to Addressee", next day service under 37 C.F.R 1.10 on the date indicated above and such envelope is addressed to the Commissioner of Patents and Trademarks, Box 5, Washington, D.C. 20231.

Joseph Chalverus, 30,144